

**UNITED STATES DISTRICT COURT
EASTERN DISTRICT OF MISSOURI
EASTERN DIVISION**

MONSANTO COMPANY and
MONSANTO TECHNOLOGY LLC,

Plaintiffs,

v.

E.I. DU PONT DE NEMOURS AND CO. and
PIONEER HI-BRED INTERNATIONAL, INC.,

Defendants.

Case No. 09-cv-0686 (ERW)

DECLARATION OF PROFESSOR VIRGINIA WALBOT

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I. INTRODUCTION

1. I have been retained by Defendants E.I. DuPont de Nemours, Inc. and Pioneer Hi-Bred International, Inc. (collectively “Defendants”) to provide this expert declaration in support of Defendants’ Motion for Summary Judgment of Invalidity for Improper Broadening Reissue, and to respond to the July 12, 2010 Declaration of Stephen Dellaporta.

II. BACKGROUND

2. I am a Professor of Biology at Stanford University.

3. I have more than 30 years of experience in plant molecular biology, primarily using corn (*Zea mays*). I consider myself to be an expert in the field of plant gene expression, having published more than 100 peer-reviewed papers in this area. My laboratory has worked on nuclear, plastid, and mitochondrial DNA organization and gene expression. We developed many of the initial vectors used for gene expression in monocots, particularly corn and rice, and we have investigated features of gene constructs that contribute to high or low expression using components derived from viruses, bacterial genes, plant genes (native to the species or from other plants), and synthetic sequences. We have tested such constructs in transient assays and in transgenic plants.

4. My laboratory has been instrumental in defining the transcription start sites, 5’ untranslated regions, intron splice sites, and 3’ untranslated regions (including poly(A) addition signals) in maize genes. Initially our contributions were through detailed analysis of these structures from maize and heterologous sources in transient assays in maize protoplasts. We discovered sequences (from heterologous organisms or synthetic sequences) that could substitute for native corn elements in supporting transcription, nuclear RNA processing and export, and efficient translation. Subsequently, my laboratory has made extensive contributions in the genomics era of maize gene discovery and definition of transcription unit properties.

5. I coordinated a 13 million dollar project funded by the National Science Foundation (NSF), called The Maize Gene Discovery Project, which over the course of 1998–2003 sequenced more than 200,000 maize Expressed Sequence Tags and more than 100,000 *RescueMu* transposon insertion sites. These data were fundamental in the elucidation of the nature of corn genes. Subsequently, I was co-principal investigator on a ~\$5 million NSF-funded project in 2004–2007 to conduct full-length cDNA sequencing from the inbred line B73; we produced finished transcript definitions for 27,750 maize genes and nearly finished definitions for an additional 5,000 genes.

6. With these data we could conduct the first public, comprehensive analysis of transcription start sites, 5' untranslated regions, context of the translational start codon, splice sites, and 3' untranslated regions for ~80% of the total nuclear gene number of maize. My laboratory has also spent nearly 30 years studying the maize transposable element *MuDR/Mu*, which upon insertion into genes dispersed throughout the genome, can disrupt or alter maize gene expression.

7. My laboratory has analyzed in detail several *Mu* insertion site cases to better understand the precise requirements for transcription and translation of maize genes, using both transient assays and transgenic plants. Our knowledge of *Mu* biology and of vector construction helped us build the *RescueMu* vector, that disrupts a color gene of maize, to do fundamental research on *Mu* excision and insertion as well as to use ~46,000 transgenic plants carrying mobile *RescueMu* elements to conduct extensive mutagenesis of maize genes in the Maize Gene Discovery Project.

8. My graduate training was in plant development and nucleic acid biochemistry at Yale University; I received my M. Phil. in 1969 and Ph.D. in 1972 from Yale University. I was

supported by a competitive fellowship from the NSF for most of my graduate training. I continued my training as a postdoctoral fellow of the National Institutes of Health in the Department of Biochemistry at the University of Georgia. There I initiated studies in mRNA processing and genome organization in cotton.

9. A copy of my Curriculum Vitae detailing my awards, appointments, and publications authored within the last ten years is attached as Exhibit A. If asked, I will testify about my research, awards, and other accomplishments as set forth in my Curriculum Vitae.

III. COMPENSATION

10. I am being compensated for my work on this case at an hourly rate of \$475, which is my standard current rate for consulting work. I have no financial interest in, and my fee is not contingent on, the outcome of this case.

IV. PRIOR TESTIMONY

11. During the past four years, I have not testified as an expert witness by deposition or trial in any matters.

V. MATERIALS CONSIDERED

12. In forming my opinions and preparing this Declaration, I have reviewed and relied upon the materials cited and listed in Exhibit B attached to this report. I have also relied on my personal knowledge, years of education, research, and experience in different fields of biological sciences, including molecular biology, biochemistry, gene expression, gene regulation, evolution, and genetics.

13. I cite various articles, patents, and other documents in my report. If called to testify, I will discuss the dates on the face of these documents (for brevity, in this report I may not repeat or discuss the dates of each article, patent, and/or documents cited). I may also use certain graphic and/or demonstrative materials to illustrate my testimony at trial.

14. For reference, I reproduce '435 Patent claim 4 and '247 Patent claim 116 below:

'435 Patent Claim 4	'247 Patent Claim 116
<p>A recombinant, double-stranded DNA molecule comprising in sequence:</p> <ul style="list-style-type: none"> a) a promoter which functions in plant cells to cause the production of an RNA sequence; b) a structural DNA sequence that causes the production of an RNA sequence which encodes a EPSPS enzyme having [four specific] sequence domains[; and] c) a 3' non-translated region which functions in plant cells to cause the addition of a stretch of polyadenyl nucleotides to the 3' end of the RNA sequence; <p>where the promoter is heterologous with respect to the structural DNA sequence and adapted to cause sufficient expression of the encoded EPSPS enzyme to enhance the glyphosate tolerance of a plant cell transformed with the DNA molecule.</p>	<p>A glyphosate-tolerant plant comprising a DNA sequence encoding an EPSPS enzyme having the sequence of SEQ ID NO:70.</p>

VI. DISCUSSION

15. I have read the Declaration of Dr. Stephen Dellaporta, dated July 12, 2010, in which he makes a number of general statements about gene expression in plant cells and offers opinions that certain things are impossible. I have also read the three articles that he cites to support his opinions. While the scientific method does not allow a definitive conclusion that something is impossible, I disagree with Dr. Dellaporta's generalizations concerning many topics, because there is a robust scientific literature disputing his conclusions.

16. I note that many of Dr. Dellaporta's specific assertions are incorrect. For instance, research conducted by my own laboratory, and reported in the literature by 1990, showed that a 3' non-translated region ("3' NTR") that polyadenylates is not always necessary, and can be substituted with other 3' NTRs that do not polyadenylate, even outside of a chloroplast. In Gallie & Walbot 1990 at 1149 (attached to the Reply Statement of Facts filed

herewith (“Reply SOF”) as Exhibit 1), we explained (emphasis added): “The 3′-UTR [i.e., 3′ NTR] of TMV [i.e., tobacco mosaic virus] was equal to or greater than a polyadenylated tail in enhancing gene expression in electroporated dicot and monocot protoplasts. The TMV 3′-UTR is functionally similar to a polyadenylated tail in that it increases mRNA stability and translation.”

17. Of greater importance, Dr. Dellaporta’s broad conclusions are incorrect because he does not take account of a substantial body of scientific evidence which does not support his theories. For instance, from what Dr. Dellaporta wrote, and from the references he cited, it is clear that he is referring only to expression of genes stably incorporated in the *nucleus* of a plant cell, and then only generally. (See Dellaporta Decl. ¶ 8 (discussing inserting “into the chromosome of a plant cell”); ¶ 10 (stating that transcription occurs inside the cell’s nucleus”); ¶ 21 (stating that the poly(A) tail “is required for the nuclear export . . . of mRNA”).

18. Dr. Dellaporta’s statements and conclusions certainly do not hold true when one considers expression of genes that are present in the DNA of a plant cell *plastid*. As discussed below, expression of plastid genes does not follow the same rules as expression of nuclear genes found in a plant cell.

19. Specifically, the arguments that the CP4 bacterial promoter could not be used to cause transcription in a plant (Dellaporta Decl. ¶¶ 15-19) and that polyadenylation is essential for plant gene expression (*Id.* ¶¶ 20-24) are overly simplistic, and only make any sense because he is talking exclusively about gene expression *in the nucleus* of a plant cell. Furthermore, the arguments make no sense and are unsupportable if one considers expression of genes in the plastid of a plant cell. In plastids, bacterial promoters *do* work to direct transcription of DNA to RNA, and polyadenylation of this RNA is not required for effective translation to protein.

20. The inventors of the ‘247 Patent understood certain benefits of inserting the DNA

for an EPSPS enzyme in the genome of a plant chloroplast as an alternative to nuclear transformation for making glyphosate-tolerant plants. In the “Statement of the Invention” section of the specification, the inventors expressly disclose plastid transformation:

While transformation of the nuclear genome of plants is much more developed at this time, a rapidly advancing alternative is the transformation of plant organelles. The transformation of plastids of land plants and the regeneration of stable transformants has been demonstrated. Transformants are selected, following double cross-over events into the plastid genome, on the basis of resistance to spectinomycin conferred through rRNA changes or through the introduction of an aminoglycoside 3'-adenyltransferase gene, or resistance to kanamycin through the neomycin phosphotransferase NptII. DNA is introduced by biolistic means or by using polyethylene glycol. This transformation route results in the production of 500-10,000 copies of the introduced sequence per cell and high levels of expression of the introduced gene have been reported. ***The use of plastid transformation offers the advantages of not requiring the chloroplast transit peptide signal sequence to result in the localization of the heterologous Class II EPSPS in the chloroplast*** and the potential to have many copies of the heterologous plant-expressible Class II EPSPS gene in each plant cell since at least one copy of the gene would be in each plastid of the cell.

(‘247 Patent, col. 33:20-46) (emphasis added; internal citations omitted). Given this disclosure in the patent of plastid transformation one would expect claims directed to glyphosate-tolerant plants to cover plants with a transgene inserted into the nucleus *or* into the chloroplast (or other plastid present in plant cells).

21. Monsanto has also published on the expression of genes in the chloroplast. (*See, e.g.,* Staub 2000 (“chloroplasts are a highly efficient vehicle for the potential production of pharmaceutical proteins in plants”) (Reply SOF Ex. 2); Ye 2001 (Reply SOF Ex. 3)).

A. Plastids

22. Indeed, glyphosate tolerance is achievable through chloroplast transformation. Scientists have been able to produce tobacco plants that are resistant to high concentrations of glyphosate by stable integration of an EPSPS gene into the chloroplast DNA of tobacco cells. (*See* Daniell 1998 (Reply SOF Ex. 4)). This technology is applicable to any type of plant,

including soybeans, corn, cotton, and canola. A brief discussion is provided to help the Court understand the biology behind the technology.

23. Plant cells typically have a rigid cell wall that surrounds the living part of the cell, called the protoplast. The protoplast consists of the plasma membrane that defines the limits of the living cell, and the cytoplasm, within which is found the nucleus. The nucleus is a membrane-bound compartment perforated with nuclear pores, and it contains the nuclear DNA, which encodes most of the genes of the organism.

24. In addition to a nucleus, the cytoplasm of plant cells contains other membrane-bound compartments, called organelles: vacuoles, mitochondria, peroxisomes, endoplasmic reticulum, Golgi bodies, plastids, etc. Of these organellar types, the plastids show a great diversity of form and function, depending on what tissue and cell types are analyzed. There are several different kinds of plastids in the cells of higher plants, defined by their morphology, biochemical capabilities, and internal structures. Some plastids found commonly in higher plants are chloroplasts, chromoplasts, leucoplasts, amyloplasts, and proplastids.

25. In developing leaves, stems, and some floral parts, the proplastids are stimulated by light to proliferate from a few proplastids per cell to many, and then this large population of dozens of proplastids differentiate into chloroplasts. Green plant tissues have this color, because the chloroplasts are green. Chloroplasts serve important functions in plant cells. Chloroplasts are the major site of photosynthesis and contain the green pigment, chlorophyll, and the other components necessary for the cell to convert light into useable high energy molecules. The chloroplast is also a location for the biosynthesis of certain amino acids that are essential for life. (See '247 Patent, col. 28:66–29:1 (“The glyphosate target in plants, the 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS) enzyme, is located in the chloroplast.”); Raven & Evert 1981 at

18-26 (Reply SOF Ex. 5)).

26. Plastids, such as chloroplasts, contain their own DNA. This plastid DNA is independent from the chromosomal DNA in the nucleus. The nuclear DNA in plant cells, formed through sexual reproduction, is a combination of DNA donated from both the male (pollen donor) and the female (ovule donor) parents. By contrast, the DNA in a plastid of a plant cell is typically inherited only from the female parent, passed from maternal parent through the egg cell. There are a few exceptional species in which both sperm and egg can transmit plastids; such biparental inheritance species typically show a large bias for maternal inheritance, that is the majority of the plastids in the offspring are from the female parent, but there are also a few paternally transmitted plastids as well. Because transgenes in plastid DNA of nearly all flowering plants (including nearly all crop species) do not get transmitted through pollen, chloroplast transformation is an important tool in restricting the dispersal of the transgene if escape of the transgene through cross pollination with other species is an environmental concern.

27. Plastid DNA includes genes that are only expressed (transcribed and translated) within the plastid. Over the past 30–40 years scientists have studied gene expression of plastid genomes utilizing both single-cell green algae and higher plants, and have found a number of mechanistic differences compared with nuclear plant gene expression. In general, it has been found that the gene expression mechanisms in plastids resemble bacterial (prokaryotic) gene expression much more than the gene expression of the nuclear DNA in eukaryotic cells. This, and other scientific evidence, has led to the conclusion that plastids evolved as free-living prokaryotes (bacteria) that then later inhabited larger, nucleated (eukaryotic) cells that were less efficient at creating energy and food. This symbiotic relationship gave the larger cells an advantage over competing cells, which eventually led to the evolution of the advantaged cells

into the modern eukaryotic plant cells containing the plastids derived from bacteria. Throughout this evolutionary process, plastids lost many genes; however, the genes remaining in the plastid genome retained many of the characteristics of a prokaryotic gene expression. (See Sugiura 1998 at 440 (“The translational machinery in chloroplasts was believed to be similar to that in *E. coli*.”) (Reply SOF Ex. 6)).

28. In one respect, genes in the chloroplasts of plant cells resemble bacterial genes in that the messenger RNA neither contains nor requires a poly(A) tail for high levels of expression. (See *id.* at 448 (“Mature and functional mRNAs in chloroplasts have no cap-structures ***nor poly(A) tails***, as found in cytoplasmic mRNAs.”) (emphasis added)). Instead of having signals that function to cause polyadenylation, most chloroplast genes contain sequences often generally referred to as 3' transcription termination regions that provide the signal for transcription termination. Rather than being polyadenylated, these regions of RNA fold into specific structures (e.g., stem-loops) that serve to protect the mRNA from being degraded. In fact, when something akin to a poly(A) tail¹ is added to the 3' end of a chloroplast mRNA, it promotes rapid degradation of the RNA, not stability and translation. (See Lisitsky 1996 at 17648 (Reply SOF Ex. 7); Lisitsky 1997 (Reply SOF Ex. 8)).

29. An illustrative example of a chloroplast gene that is not polyadenylated is the gene for the S16 protein from the chloroplast genome of potato cells. In an article describing the nucleotide sequence of this gene, the authors state:

The 3' end of the untranslated sequence consists of 146 bp and ***shows typical plastid gene construction in that it contains no polyadenylation signal***, but

¹ Chloroplast RNAs have been detected with short tails made up primarily of A and G ribonucleotides.

instead has several inverted repeat sequences, which could form a hairpin structure in their DNA and a stem-loop structure in their transcripts.

(Kang & Hannapel 1995 at 294 (emphasis added) (Reply SOF Ex. 9)). It is also mentioned that stem-loop structures are typical of plastid RNA and that the sequences responsible for the folding resemble prokaryotic transcription terminators. *Id.*

B. Glyphosate-Tolerant Plants and Plant Cells Comprising a DNA Encoding an EPSPS Enzyme Do Not Necessarily Require the DNA to Have A 3' NTR that Functions to Cause the Addition Of A Poly(A) Tail To The RNA

30. I understand that Monsanto has taken the position that it is *impossible* to produce a glyphosate-tolerant plant as a result of a DNA encoding the EPSPS enzyme of the *Agrobacterium* sp. strain CP4, unless the coding DNA is part of a gene that includes a 3' NTR that functions in plant cells to cause the addition of a stretch of polyadenyl nucleotides to the 3' end of the RNA sequence. I understand they base this conclusion on the Dellaporta Declaration.

31. However, the Dellaporta Declaration does not support Monsanto's conclusion, because Dr. Dellaporta does not address the expression of genes from the chloroplast as the inventors of the '247 Patent contemplated. A gene construct with a promoter that functions in chloroplasts (discussed further below), a ribosome binding site, a structural sequence for an EPSPS enzyme, and the 3' non-translated sequence of the potato chloroplast S16 gene would be transcribed and translated by the chloroplast of a plant cell. A plant with such a gene inserted into its chloroplasts could be glyphosate-tolerant. In fact, the 3' non-translated region from other typical chloroplast genes or bacterial genes would work to terminate transcription and stabilize the mRNA, but would not cause the RNA to be polyadenylated by the plant cell. (*See* Daniell 1998 (Reply SOF Ex. 4); Valkov 2010 at Table 1 (Reply SOF Ex. 10)).

32. Monsanto's position is further contradicted by their own publications and patents, including U.S. Patent No. 7,732,662 ("Method for the transformation of plant cell plastids")

(Reply SOF Ex. 11); U.S. Patent No. 6,492,578 (“Expression of herbicide tolerance genes in plant plastids”) (Reply SOF Ex. 12); and Ye et al., *Plastid-expressed 5-enolpyruvylshikimate-3-phosphate synthase genes provide high level glyphosate tolerance in tobacco*, Plant J. 25(3):261-270 (2001) (Reply SOF Ex. 3).

33. These references state that plants that produce the CP4 EPSPS enzyme in the chloroplasts are glyphosate-tolerant. For instance, in the Ye 2001 article, Monsanto scientists explain: “We report here the development of *very high levels of glyphosate tolerance via plastid transformation technology.*” (*Id.* at 267 (emphasis added)).

34. The gene constructs used to make these glyphosate-tolerant plants do not include a 3’ NTR that functions to cause the addition of a stretch of polyadenyl nucleotides to the 3’ end of the RNA. Instead, the constructs use transcription terminator sequences from highly expressed chloroplast genes, which do not naturally specify addition of poly(A) regions but function as transcription termination sequences in the manner of prokaryotic signals.

35. Moreover, Monsanto’s ‘662 patent contains a statement that indicates that the transcript terminator region is not an essential component of gene expression (at col. 12:24-33 (emphasis added)):

Regulatory transcript termination regions may be provided in the expression constructs of this invention as well. Transcript termination regions may be provided by any convenient transcription termination region derived from a gene source, for example, the transcript termination region that is naturally associated with the transcript initiation region. ***The skilled artisan will recognize that any convenient transcript termination region that is capable of terminating transcription in a plant cell may be employed in the constructs of the present invention.***

36. It is clear that these Monsanto inventors do not think that it is impossible to make a glyphosate-tolerant plant that produces the CP4 EPSPS enzyme without having a signal in the 3’ non-translated region that functions to cause polyadenylation, because there are transcription

terminators that function in plant cells that do not signal the addition of a poly(A) tail to RNA. I also note that Monsanto's '662 and '578 patents both incorporate by reference, into their written description, Monsanto's original '435 Patent in its entirety. (*See* '662 patent, col. 12:43-45; '578 patent, col. 2:54-58).

37. In another Monsanto patent (assigned to Calgene, a Monsanto subsidiary), U.S. Patent No. 5,576,198 ("Controlled expression of transgenic constructs in plant plastids") (Reply SOF Ex. 13), the inventors claim a system of expressing proteins in the chloroplast from constructs that have terminators from viral genes that normally infect bacteria (at col. 6:66-7:9):

The plastid viral promoter expression constructs will generally include a transcription termination region that is recognized by the viral polymerase encoded by the nuclear construct. Typically, a strong transcription termination region is required when using the specific viral promoters of this invention, and it is thus convenient to use the transcription termination region from the same gene as that from which the specific promoter region was obtained. Thus, in the examples described herein, a T7 gene 10 promoter region and the corresponding gene 10 transcription termination region are used in the plastid T7 expression constructs.

38. The transcription termination regions described in the '198 Patent also do not function to cause polyadenylation, but nonetheless are used to express an EPSPS gene to render a plant glyphosate-tolerant.

39. The technology in Monsanto's '662, '578, and '198 patents is applicable in all types of plants, including soybeans, corn, cotton, and canola. (*See* '662 patent, col. 8:3-20 ("Particularly preferred plants include" soybeans, corn, cotton, and canola); '578 patent, col. 10:14-18 ("Suitable plants for the practice of the present invention include, but are not limited to" soybeans, cotton and corn); '198 patent, col. 11:27-35 ("the constructs and methods described herein may be employed with a wide variety of plant life" including soybeans and corn).

40. Accordingly, Monsanto is wrong to conclude that it is "impossible" to produce a glyphosate-tolerant plant from an EPSPS gene lacking a 3' non-translated region ("3' NTR") that

functions in plant cells to cause the addition of a stretch of polyadenyl nucleotides to the 3' end of the RNA sequence, because Monsanto and Dr. Dellaporta did not address expression of chloroplast or other plastid genes.

C. Glyphosate-Tolerant Plants and Plant Cells Comprising a DNA Encoding an EPSPS Enzyme Do Not Necessarily Require a Heterologous Promoter, Because a Bacterial Promoter Can Be Used for Plastid Expression

41. I also understand that Monsanto has taken the position that it is *impossible* to produce a glyphosate-tolerant plant as a result of a DNA encoding the EPSPS enzyme of the *Agrobacterium* sp. strain CP4, unless the coding DNA has a promoter that is heterologous. I understand they base this conclusion on Dr. Dellaporta's opinion that bacterial promoters do not work well in plant cells.

42. Bacterial promoters, however, do work in plant cell plastids, such as chloroplasts. (See Valkov 2010 (Reply SOF Ex. 10), Newell 2003 (Reply SOF Ex. 14)).

43. Indeed, Monsanto's '198 Patent also recognizes this fact (at col. 8:10-16) (emphasis added):

In the examples described herein, the *aadA* gene is under the control of a tobacco 16S rRNA promoter, *rrn* region and a tobacco *rps16* 3' termination region. Numerous additional promoter regions may also be used to drive expression of the selectable marker gene, including various plastid promoters ***and bacterial promoters which have been shown to function in plant plastids.***

44. Monsanto's '578 patent states similarly that bacterial promoters "have been shown to function in plant plastids" (at col. 8:42-44).

45. Thus, there is no scientific basis to conclude that the promoter that naturally controls the transcription of the EPSPS gene from *Agrobacterium* sp. strain CP4 would not work to initiate transcription in the chloroplast of a plant cell.

46. I also note that transcription is not a major controlling step of gene expression in the chloroplasts. Rather, chloroplast gene expression is regulated at the translation step.

Therefore, the most important element in a chloroplast gene construct is something called a ribosome binding site (RBS). The RBS is a sequence in the RNA that allows the machinery for translation into the protein to associate with the RNA and begin the process of protein synthesis. A gene construct with an effective RBS will express high levels of protein even if the promoter is a relatively weak promoter. (*See* Mayfield 1995 (Reply SOF Ex. 15)).

47. Last, I also note that the sequence of a bacterial promoter could be modified to render it capable of functioning in a plant nucleus. For instance, the promoter from CP4 bacteria could work in the nucleus if it were modified to include cis-acting elements. Such a modified bacterial promoter would be non-heterologous when used to express a bacterial EPSPS gene. Accordingly, a non-heterologous, modified CP4 bacterial promoter could be used to express the CP4 EPSPS gene in the nucleus to make a glyphosate-tolerant plant.

D. Even in Nuclear-Transformed Genes, a Heterologous Promoter is Not Required Because a Non-Heterologous Plant Promoter Could Be Used to Express a Plant-Derived EPSPS

48. In addition, there is no reason why the DNA encoding the EPSPS enzyme of SEQ ID NO:70 must come from the CP4 bacteria. Although one *may* derive a DNA sequence encoding an EPSPS enzyme having the sequence of SEQ ID NO:70 from DNA found in the CP4 bacteria, one need not—a DNA sequence obtained that way is only *one of many ways* of obtaining such a DNA. Given the redundancy of the genetic code (i.e., the fact that numerous codons can specify the same amino acids), there are many millions of different DNA sequences that encode an EPSPS enzyme having the sequence of SEQ ID NO:70.

49. It is certainly within the realm of scientific possibility that a plant has (or will have) a gene that encodes an EPSPS enzyme of SEQ ID NO:70. That such a gene has not yet been found in a plant does not mean that it does not exist, or that it cannot exist. Given the immense variety and diversity of plants, it is conceivable that one could be isolated from a

naturally existing plant; indeed, naturally glyphosate-tolerant plants with distinct EPSPS genes have been reported. (*See* Yuan 2002 (Reply SOF Ex. 16)).

50. One also could, through selection, obtain a plant with an EPSPS gene encoding SEQ ID NO:70. The appearance of glyphosate-tolerant weeds shows the ability for plant species to adapt to evolutionary pressure. A plant EPSPS gene could also be obtained by way of mutagenesis in a laboratory, e.g., by exposing plants to chemicals or radiation. This is a possibility the '247 Patent itself discusses. (*See* '247 Patent, col. 1:62-64 ("A number of glyphosate-tolerant plant variant EPSPS genes have been constructed by mutagenesis.")).

51. Moreover, I understand that the DNA sequence that would encode an EPSPS enzyme having the sequence of SEQ ID NO:70 discussed in the '247 Patent was in fact not directly obtained from the CP4 bacteria. SEQ ID NO:70 is an "artificial sequence," engineered by modifying the natural DNA sequence in a way that changes the protein that will be encoded. (*See* '247 Patent, cols. 29:13-23, 42:27-30; *id.*, col. 153 (describing SEQ ID NO:70 as "ORGANISM: Artificial sequence . . . OTHER INFORMATION: Synthetic")). A scientist could clone any plant EPSPS gene along with its natural promoter sequences, and then modify the plant EPSPS gene in the laboratory so that it encodes SEQ ID NO:70. Specific changes to the plant EPSPS gene also could conceivably be rendered using a method such as gene targeting to substitute in a modified EPSPS gene as a replacement for the endogenous native gene.

52. For example, one could start with the EPSPS cDNA from *Petunia hybrida* and perform multi-round site-directed mutagenesis in order to derive from it a sequence corresponding to CP4 EPSPS. First, one could determine which nucleotides one needs to mutate within the petunia cDNA so that the mutated codons of the open reading-frame correspond to the amino acid sequence of CP4 EPSPS. For each round, one would then mutate about ten

nucleotides, spaced evenly throughout the petunia EPSPS cDNA. After each round, one would sequence the mutated cDNA and verify that the desired changes had been made. Once verified, one would use this mutated cDNA as starting material for the next round, introducing another ten evenly spaced mutations, and so on, repeating this process until all of the desired changes had been introduced into the petunia cDNA.

53. Thus, it is perfectly conceivable that a DNA sequence that encodes an EPSPS enzyme having the sequence of SEQ ID NO:70 could be either obtained directly from a plant or derived from a plant gene through modification. In either situation, the homologous promoter (i.e., the promoter native to that plant EPSPS DNA sequence) would work to cause expression of the EPSPS protein in the nucleus of a plant cell. In other words, a non-heterologous plant promoter could be used with a plant EPSPS DNA.

VII. FURTHER TESTIMONY

54. If requested by the Court I may provide oral testimony at a hearing or at trial consistent with the statements made in this declaration.

55. These opinions are based upon the information I have reviewed to date. I expressly reserve the right to continue my investigation including review of documents and information that may yet be produced and not yet available or any other information provided in this action, including at trial. Therefore, I reserve the right to expand, modify, supplement, or amend my opinions in response to any additional information that becomes available to me and opinions expressed by Monsanto's experts or experts representing a third party, or in light of any additional evidence, testimony, or other information, including any further claim construction that the Court may make.

I declare under penalty of perjury under the laws of the United States that the foregoing is true and correct to the best of my knowledge and carefully considered opinion.

Dated: August 11, 2010
Stanford, California



Virginia Walbot, Ph.D.

CERTIFICATE OF SERVICE

I hereby certify that on August 11, 2010, I electronically transmitted the foregoing document to the Clerk of Court using the ECF System for filing and transmittal of a Notice of Electronic Filing to the following ECF registrants:

- **Steven M. Berezney**
steve.berezney@huschblackwell.com

- **Kurt G. Calia**
kcalia@cov.com

- **Matthew A. Campbell**
macampbell@winston.com

- **Scott W. Clark**
sclark@mwe.com

- **Joseph P. Conran**
joe.conran@huschblackwell.com

- **Todd J. Ehlman**
tehlman@winston.com

- **Anthony J. Franze**
Anthony.Franze@aporter.com

- **Greg G. Gutzler**
greg.gutzler@huschblackwell.com

- **James M. Hilmert**
jhilmert@winston.com

- **George C. Lombardi**
glombardi@winston.com

- **Kurt A. Mathas**
kmathas@winston.com

- **Omri E. Praiss**
omri.praiss@huschblackwell.com

- **John J. Rosenthal**
jrosenthal@winston.com

- **Rebecca M. Ross**
rmross@winston.com

- **Mark A. Smith**

markasmith@winston.com

- **Steven G. Spears**

sspears@mwe.com,jjknapp@mwe.com

- **Tamara M. Spicer**

tamara.spicer@huschblackwell.com,jean.melenbrink@huschblackwell.com

- **Gail J. Standish**

gstandish@winston.com

- **Dan K. Webb**

dwebb@winston.com

/s/ C. David Goerisch